APPLICATION OF A NOVEL STATISTICAL MODEL FOR QUANTITATIVE PROTEOMICS BY ^{18}O LABELING TO THE STUDY OF VEGF-INDUCED ANGIOGENESIS IN VASCULAR ENDOTHELIAL CELLS

Pablo Martínez-Acedo[1], Pedro Navarro[1], Inmaculada Jorge[1], Estefanía Nuñez[1], Horacio Serrano[1], Arántzazu Alfranca[2], Juan Miguel Redondo[2] and Jesús Vázquez[1]

[1]Centro de Biología Molecular Severo Ochoa, Madrid, Spain;
[2]Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain

Quantitative proteomics, which may be defined as the study of global changes in the expression level of proteins, is a field that has experimented a great development in the last years. While a great effort has been devoted to the development of bioinformatics tools for automated analysis of MS data and calculation of peptide ratios for quantitative proteomics approaches using each one of the several isotope labeling strategies currently available, existing analytical methods for the statistical determination of significant expression changes are scarce. In fact**,** no specific statistical models have still been proposed to deal with data produced by enzymatic $^{16}O/^{18}O$ labeling.

We have developed a hierarchical, random-effects model including four different sources of variance at the spectrum-fitting, scan, peptide and protein levels. To validate our statistical model, we have performed a large-scale null-hypothesis experiment using the $^{16}O/^{18}O$ labeling technique on the proteome of human umbilical vein endothelial cells (HUVEC), by comparing two identical proteome extracts. Among more than 1,200, 20 proteins would have been considered as false expression changes at a FDR of 5% by applying conventional models based on the normality assumption. However, only 1 expression change was detected using the new random-effects model.

The new method was applied to the study of molecular mechanisms underlying angiogenesis in endothelium. Expression changes in the protein profile of HUVEC in culture in response to the pro-angiogenic factor VEGF were analyzed after 4- and 8-h incubation. About 2,000 proteins were identified in each experiment, among which we were able to quantify about 1,000 proteins from which 32 and 46 proteins were found to be differentially expressed, respectively. These changes included proteins implicated in protein binding, metabolism, transport and response to external stimuli. The consistency of the changes observed at 4h was confirmed by a replica at a smaller scale and further validated by Western blot analysis of selected proteins (annexin A1, reticulocalbin and triose-phosphate-isomerase). The expression pattern clearly shows two distinct populations of changing proteins indicating that the angiogenesis is a biphasic process that reflects a specific short-term response of endothelial cells to VEGF.